Stressor interactions under differential exogenous microbial exposure in *Daphnia magna*

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**Abstract**

Studies on stressor responses are often performed in controlled laboratory settings. The microbial communities in laboratory setting often differ from the natural environment, which could ultimately be reflected in different stress responses. In this study, we investigated how stressor responses differed between laboratory and natural conditions in *Daphnia magna* when exposed to single or multiple stressors. *Daphnia* individuals were exposed to the toxic cyanobacterium *Microcystis aeruginosa* and a fungal infection, *Aspergillus aculeatus* like type. Three genotypes were included to investigate genotype-specific responses. Survival, reproduction and body size were monitored for three weeks and gut microbial communities were sampled and characterized at the end of the experiment. Our study shows that natural environments have a more diverse microbial community compared with laboratory conditions, which was ultimately reflected in the gut microbiomes after inoculation. Stressor responses in *Daphnia* were affected by their bacterial environment for survival, but not for fecundity and body size. Fecundity and body size did show
a main stressor effect, which could possibly be linked with stessor-specific microbiomes (for *Microcystis* and the combined stessor treatment). In addition, genotype-specific responses were detected for survival and fecundity, which could be linked with the selective capabilities of the *Daphnia* genotypes to select beneficial or neutral microbial stains from the environment.

**Introduction**

Organisms are constantly involved in biotic and abiotic interactions that lead to the flourishment and diversity of life (Bøhn and Amundsen 2004, Ratzke et al. 2020). Interactions with environmental and biotic stressors have an important role in shaping co-evolutionary dynamics by altering the strength of and response to selection and/or population dynamics (Theodosiou et al. 2019, Thompson and Cunningham 2002). We can fairly say that all organisms in their natural settings are forced to cope with environmental stress (Holmstrup et al. 2010). There is, however, increasing evidence that *single stressors generally co-occur* and interact (Jackson et al. 2016) and generate complex effects on natural populations (Piggot et al. 2015). A meta-analysis by Jackson et al. (2016) of the marine literature shows that the net impact of multiple stressors are frequently either greater than (i.e. a synergistic interaction) or equal to (i.e. an additive effect) the sum of their single effects. Net effects of two or more stressors that were less effective than the potential additive outcome (i.e. antagonistic interaction) are less common in marine systems, but occur frequently in freshwater systems (Crain et al. 2008, Holmstrup et al. 2010, Jackson et al. 2016). One important aquatic stressor is cyanobacteria which is becoming increasingly dominant in aquatic ecosystems (Visser et al. 2016). The negative effect of cyanobacteria on zooplankton is well
documented (Ferrão-Filho et al. 2000, Asselman et al. 2012, Lemair et al. 2012). Cyanobacteria are known to produce a wide range of toxic, secondary metabolites, classified as cyanotoxins among which hepatotoxins, neurotoxins, dermatotoxins, and general cyanotoxins (De Figueiredo et al. 2004, Bittner et al. 2021). A commonly occurring and well-studied cyanobacterium is *Microcystis* sp. (von Elert et al. 2003), which is known to produce various toxins, such as the most-frequently occurring hepatotoxic microcystin (Van appeldoorn et al. 2007). The toxic effects of microcystins are detrimental for the survival and health of aquatic organisms such as zooplankton and fish (Penaloza et al. 1990). *Microcystis* is also of low food quality due to the absence of essential polyunsaturated fatty acids and sterols (von Elert et al. 2003, Martin-Creuzburg et al. 2008). Additionally, *Microcystis* is known for its colony formation which interferes with the filtering process (DeMott et al. 2001), which further negatively impacts zooplankton fitness. In response, zooplankton has developed multiple anti-*Microcystis* defences in e.g., production of proteases or increased gene expression of genes associated with secondary metabolite transport and catabolism (Schwarzenberger et al. 2014).

Not only cyanobacteria pose a severe threat to the zooplankton communities, also parasites are an increasing threat, especially upon global change. Fungal parasitism received increasing scientific interest in the last years (for zooplankton: e.g. Decaestecker et al. 2005, Civitello et al. 2015, Banos et al. 2020; for cyanobacteria: e.g. Gerphagnon et al. 2015, Gleason et al. 2015) and are omnipresent and diverse in morphology, phylogeny and ecological functions. Fungal parasites, however, are still poorly understood in their role in vital interactions and ecosystem functions in most aquatic ecosystems (Grossart et al. 2019). Combined with the increasing abundance of cyanobacterial blooms, this sparked some studies to examine potential interactions between fungi
and cyanobacteria impacting aquatic food webs. Some research focussed on altered predator-prey interactions by fungal infections of cyanobacterial blooms (e.g. Kagami et al. 2007, Tao et al. 2020). Agha et al. (2016) focussed on chytrid infection of cyanobacterial populations, revealing a positive impact on the zooplankter _Daphnia_ by improving food quality. Other research focussed on altered host-parasite interactions by feeding infected _Daphnia_ populations with cyanobacteria (Coopman et al. 2014, Boudry et al. 2020). Boudry et al. (2020) revealed an antagonistic interaction between a fungal parasite and _Microcystis_ as a higher survival was obtained in infected _Daphnia_ compared with non-infected _Daphnia_ when fed on _M. aeruginosa_. Other studies have also revealed antagonistic interactions using other parasitic systems in _Daphnia_ (e.g. predation x bacterium: Coors and De Meester 2008, pesticide x bacterium: De Coninck et al. 2013, salinity x bacterium: Hall et al. 2013, cyanobacteria x iridovirus: Coopman et al. 2014, microsporidium x bacterium: Lange et al. 2014).

The last decade, studies have shown that it is not just the host’s genome that determines host fitness and reaction towards stressors, but rather a complex interplay of the host genome and microbiome (McFall-Ngai et al. 2013). Especially the gut microbiome, the genetic material of all microorganisms present in the host’s gut, plays a key mediating role in host physiology (e.g. organ development: McFall-Ngai et al. 2013, immunoregulation: Renz et al. 2011, metabolism: Turnbaugh et al. 2006). Research has shown that the microbial community in _Daphnia_ is structured by diet (Callens et al. 2016), host genetics (Macke et al. 2017, 2020, Bulteel et al. 2021), antibiotics (Callens et al. 2018, Motiei et al. 2020), temperature (Sullam et al. 2018, Frankel-Bricker et al. 2020) and cyanobacterial exposure (Macke et al. 2017). Mushegian et al. (2018) suggested that _Daphnia_ functioning is largely determined by environmental bacteria, suggesting a strong role of
horizontally transmitted symbionts. Callens et al. (2020) showed that exogenous exposure to
different environmental pools of bacteria, resulted in different gut microbial communities,
reflected in both community composition and community structure. These results show an
important role of the bacterioplankton community in structuring the gut microbial community in
*Daphnia*. As *D. magna* is a well-established and key study system, many studies have been
performed on this model organism, but mostly under laboratory conditions. The bacterioplankton
community under laboratory conditions, however, differs from communities in natural
conditions, among which a reduced species richness in the laboratory communities (Callens et al.
2020). Similar studies on fish and mice have shown that the gut microbiome from hosts in
laboratory conditions are to some extent the same, but also differ from its free-roaming
counterpart under natural conditions, which may modulate a different response to inflammatory

In general, exposure to different bacterial environments could impact the strength and specificity
of stressor responses (e.g. host-parasite: Wolinska and King 2009). Host organisms under
laboratory conditions encounter fewer microbes compared with their free-roaming counterparts,
which should ultimately be reflected in a less diverse laboratory host microbiome. Previously, it
has been shown that invasion of pathogens decreases when soil bacterial diversity is high (van
Elsas et al. 2012). Booth (2002) has also shown that bacterial heterogeneity could aid in the survival
of the host, whereby a small fraction of the bacterial population would be able to survive the
exposure to single or multiple stressors that kill the majority of the population. These studies
indicate that high bacterial diversity is a codetermining factor in protecting the host against single
or multiple stressors. As the host microbiome plays a crucial role in immune responses, exogenous
exposure to laboratory microbiota could potentially not mirror expected tolerances as occurring in natural populations (Greyson-Gaito et al. 2020). With this experiment we aim to investigate the response of *D. magna* individuals to single or multiple stressors when exposed to different exogenous microbial inocula. Individuals, inoculated with either a natural or a laboratory microbial community, were exposed to one of the four stressor treatments: the toxic cyanobacterium *M. aeruginosa* (further referred to as *Microcystis*), infection with the fungus *Aspergillus aculeatus* (further referred to as *Aspergillus* or infection), the combination of both *M. aeruginosa* and the infection (further referred to as Combi), and a control treatment (fed with *Chlorella vulgaris* instead of *Microcystis* and no exposure to the infection).

Firstly, we expect an impact of the microbial exposure on *Daphnia* life history responses when comparing the stressor treatments. We hypothesize that individuals receiving the natural microbial inoculum will have a higher tolerance to particular stressors (i.e., have a higher survival, fecundity and body size) compared with individuals that receive a laboratory microbial community as natural bacterioplankton communities are generally associated with a more diverse microbial community and provide a broader pool of microbiota for the host to select beneficial strains from. In addition, we hypothesize that exposure to the natural microbial community will result in a lower additive outcome of the antagonistic interaction (antagonistic, as described in Boudry et al 2020) compared with exposure to a lab microbial community. A reduced effect of the single stressors on the individuals, will consequently result in a lower net effect of the multiple stressor as we assume a higher tolerance towards single stressors when exposed to a natural microbial community. Finally, we include the role of the host genotype as previous research has revealed a strong genotype-effect on the present gut microbial community and genotype x
microbiome interactions with respect to stress tolerance (Macke et al. 2017, 2020, Callens et al. 2020, Massol et al. 2020, Bulteel et al. 2021, Houwenhuyse et al. 2021), so we expect intraspecific differences within *D. magna* responses to the stressors under the different exogenous microbial exposures.
## Results

Table 1: Overview results LMER for life history traits for the recipients and amplicon sequencing for the combination of donors and recipients, the donors separately and the recipients separately. Significant results (p<0.05) are indicated with *. Highly significant results (p<0.001) are indicated with **. df = degrees of freedom.

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<th>Survival Chi²</th>
<th>Fecundity p-value</th>
<th>Fecundity F</th>
<th>Body Size p-value</th>
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<th>OTU richness p-value</th>
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<th>Beta diversity p-value</th>
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The survival analysis revealed a main genotype effect, microbiome x genotype interaction, and stressor x microbiome x genotype interaction on *Daphnia* survival. No stressor x microbiome interaction was present. Separate analyses per microbiome treatment, however, revealed a significant main effect of the stressor treatment in *Daphnia* individuals that received a laboratory microbial inoculum ($X^2=9.5$, df=3, $p=0.02$), but not in *Daphnia* that received a natural microbial inoculum ($X^2=0.8$, df=3, $p=0.9$). In the lab microbial treatment, *Daphnia* that received a *Microcystis* treatment had a significant lower survival than *Daphnia* individuals that received the combi ($X^2=6.9$, df=1, $p=0.009$; Figure S1) and the control treatment ($X^2=4.9$, df=1, $p=0.03$; Figure S1). Genotype, additionally, determined survival as our analyses revealed a significant stressor x microbiome x genotype interaction (Table 1, Figure 1). When tested separately per genotype, no significant stressor x microbiome interaction was revealed for KNO, OM2 or T8 individuals (Table S1). We did, however, find a significant stressor x genotype interaction in *Daphnia* receiving a natural microbial inoculum ($X^2=22$, df=11, $p=0.02$), but not in *Daphnia* receiving a laboratory microbial inoculum ($X^2=14$, df=11, $p=0.2$). Within the natural microbial treatment, the survival probability of the T8 individuals was significantly lower compared with KNO individuals ($X^2=5.6$, df=1, $p=0.02$; Figure 1) and OM2 individuals ($X^2=7$, df=1, $p=0.008$; Figure 1) for the control treatment. For all the other stressor treatments within the natural microbial treatment, no significant differences between the genotypes were observed (Table S1).
Figure 1: Survival plots of recipient Daphnia between the stressors treatments for the different microbial inocula (columns) and genotypes (rows). Colors indicate the different stressor treatments.

**Total fecundity**

The fecundity analysis revealed a significant main effect of the stressor treatment and genotype, a significant stressor x genotype interaction, and stressor x microbiome x genotype interaction on total fecundity (Table 1). Analyses revealed no significant effect of stressor x microbiome on total fecundity (Table 1, Figure S3). Separate analysis per microbiome treatment, revealed a significant main effect of the stressor treatment in Daphnia individuals that received a natural (F=47.36, df=3, p<0.0001) and laboratory microbial inoculum (F=15.53, df=3, p<0.001), with total fecundity
significantly differing between the control treatment and the *Microcystis*, and control and combi
treatment. On average, *Daphnia* had a lower reproduction when they received *Microcystis* (both as
a single stressor and in the combi treatment) compared with the control and infection treatment
(Figure S2). Genotype co-determined total fecundity as our analyses revealed a significant stressor
x microbiome x genotype interaction (Table 1, Figure 2). The KNO genotype revealed significant
differences between the infection and *Microcystis* treatment, and the infection and combi
treatment within both microbial inocula (Table S1, Figure 2). The OM2 genotype revealed
significant differences for fecundity between the control and *Microcystis*, infection and *Microcystis*,
and infection and combi when exposed to the natural microbial inocula (Table S1, Figure 2). A
similar pattern was observed within the laboratory microbial inocula for OM2 with an additional
significant difference between the control and combi treatment (Table S1, Figure 2). The T8
genotype revealed no significant differences between the stressor treatments within both
microbial inocula (Figure 2).
Figure 2: Box plots of the total brood for the different stressor treatments for the three-way interaction (stressor x microbiome x genotype). Box plots are given for the two microbiome treatments (columns) and the three genotypes (rows). Colors indicate the different stressor treatments.

Body size

Analyses on Daphnia body size revealed a significant main effect of the stressor treatment (Table 1, Figure 3). Post hoc analyses showed a significant difference between all stressor treatments, except between the single stressor Microcystis and the combi treatment (Table S1). Individuals in the control treatment had the highest body size, followed by, in decreasing order of body size, individuals exposed to the infection, Microcystis and the combi treatment (Figure 3). No impact of microbiome treatment or genotype were detected for Daphnia body size (Table 1).
Figure 3: Box plots of the recipient body size at the end of the experiment per stressor treatment. Colors indicate the different stressor treatments.

**Microbial composition**

**Microbial community**

Combined donor and recipient microbial communities were dominated by Betaproteobacteriales (mean=46.484%, sd=26.554%), Pseudomonadales (mean=20.005%, sd=23.323%) and Verrucomicrobiales (mean=5.388%, sd=7.092%). Donor microbial communities, analyzed separately, were dominated by Betaproteobacteriales (mean=33.092%, sd=22.393%), Micrococcales (mean=21.3728%, sd=31.5502%) and Chitinophagales (mean=11.525%, sd=17.676%), whereas
recipient microbial communities were dominated by Betaproteobacteria\(l\)es (mean=48.397\%, sd=26.780\%), Pseudomonadales (mean=22.464\%, sd=23.944\%) and Verrucomicrobiales (mean=5.811\%, sd=7.317\%, Figure 4). A similar top 3 was observed for all recipient groups, whether they were exposed to the laboratory or natural microbial inoculum. Additionally, the same top 3 was observed for recipient Daphnia, indifferently of the stressor treatment, except for Daphnia exposed to the control, whereby the third most abundant order was Rhizobiales instead of Verrucomicrobiales (Table S2).

To examine a possible link between the gut microbial community and the life history traits, Union plots were performed for the stressor x microbiome interaction. When comparing the total number of OTUs from Daphnia exposed to the control treatment with the single stressor treatments (Figure 5A and 5B), a higher number of OTUs in the single stressor treatments (Infection: n=153, Microcystis: n=156) was observed compared with the control treatment (n=134) within the laboratory microbial inocula. The opposite was observed within the natural microbial inocula, whereby Daphnia exposed to the single stressor treatments (Infection: n=196, Microcystis: n=183) had a lower total number of OTUs compared with the control treatment (n=202). Union plots comparing the single and multiple stressor treatments (Figure 5C and 5D) showed that the total number of OTUs was lower in the combi treatment (lab: n=138, natural: n=167) compared with the infection treatment (lab: n=154; natural: n=186) and the Microcystis treatment (lab: n=161; natural: n=170) for both microbial inocula.
Figure 4: Relative abundance of the gut microbial composition of the recipient population grouped per genotype x microbiome x stressor interaction. Colors indicate the bacterial order. OTUs with a relative abundance lower than 1% are not included. Analyses are performed on rarefied data.
Figure 5: Union plots representing the OTUs that are unique within and shared between stressor treatments when exposed to the lab (A and C) or natural microbiome inocula (B and D). OTUs illustrated in A and B are: control (C), infection (I) and *Microcystis* treatment (M). OTUs illustrated in C and D are: infection (I), *Microcystis* (M) and combi treatment (MI). Numbers between brackets indicate the total number of OTUs. Colors indicate OTUs grouped per class.
Genotype T8 displayed a lower survival probability compared with KNO and OM2, when receiving a natural microbial inoculum and control treatment. To examine a possible link between survival and the gut microbial community, a Union plot for the three clones within the natural microbial inocula and control treatment was made (Figure 6A). The complementary Union plot for the laboratory microbial inocula and control treatment (Figure 6B) was also portrayed as T8 did not show this reduced survival probability under laboratory conditions. T8 had a higher number of unique OTUs (n=58) and total number of OTUs (n=117), compared with KNO (unique: n=30, total: n=85) and OM2 (unique: n=35, total: n=95) when receiving the natural inocula. When we examined the present OTUs after receiving the laboratory inocula, T8 (unique: n=32, total: n=82) had the same number of unique OTUs as KNO (unique: n=32, total: n=84) and a higher number of unique OTUs compared with OM2 (unique: n=21, total: n=70).

When comparing main stressor treatment effects, the highest number of total OTUs was observed in the gut community of individuals which received the infection treatment (n=251), followed by the control treatment (n=246) and the *Microcystis* and combi treatment (both n=243, Figure S4).
Figure 6: Union plots representing the OTUs that are unique within and shared between the genotypes in the control treatment when exposed to the (A) lab and (B) natural microbiome inoculum. Numbers between brackets indicate the total number of OTUs present in that compartment. Colors indicate the OTUs grouped per class.

EdgeR analysis revealed highly significant differences for 213 OTUs between the donor and recipient Daphnia (Table S3). Within the donors, only one OTU was highly significantly different between the laboratory and natural microbial inocula (Table S3). Within the recipients 141 OTUs were significantly different between the four stressor treatments, 285 OTUs between the microbiome treatments, 34 OTUs within the stressor x microbiome interaction and 5 OTUs were significantly different within the stressor x microbiome x genotype interaction (Table S3; Figure 7). Analysis per microbiome treatment revealed significant differences between the stressor
treatments for 12 OTUs within the lab microbiome treatment and for 24 OTUs within the natural microbiome treatment (Figure 8, Table S3).

**Figure 7: ggplot** representing the OTUs at family level that were significantly different between the stressor x microbiome x genotype interaction. Colors indicate the stressor treatments.
Figure 8: ggplot representing the OTUs at family level that were significantly different between the different stressor treatments within the (A) lab and (B) natural microbiome treatment. Colors indicate the stressor treatments.
OTU richness

Analysis of the data set containing both the microbial donor inocula and the recipient gut microbiomes revealed a significant sample type (donor vs recipient) x microbiome interaction (Table 1). Post hoc analysis revealed significant differences between all combinations, except between the laboratory donors and the natural recipients (Table S4). In both donor inocula and recipient microbiomes, OTU richness was significantly higher in the natural conditions (donor: mean= 87.000, sd= 42.036, recipient: mean= 26.550, sd= 9.556) compared with the laboratory conditions (donor: mean= 30.333, sd= 10.970, recipient: mean= 19.652, sd= 5.441, Table S4, Figure 9). OTU richness was also significantly higher in the donors (mean= 58.667, sd= 41.452) compared with the recipients (mean= 22.860, sd= 8.303; p<0.001, z-value=-12.13, Figure 9). Analysis of the recipients revealed a significant microbiome effect on OTU richness (Table 1). No stressor, stressor x microbiome interaction or stressor x microbiome x genotype interaction was observed (Table 1). A separate analysis per microbiome treatment did not reveal a significant main effect of the stressor treatment in both Daphnia individuals that received a laboratory microbial inoculum (Res. Dev. = 23.174, df=3, p= 0.129) or a natural microbial inoculum (Res. Dev. = 57.756, df=3, p= 0.056).
Figure 9: Bar plots of OTU richness of donor and recipient samples which are grouped per sample type and microbial inocula. Colors indicate the different microbial inocula. Error bars indicate standard error.

**Beta diversity**

Analysis on beta diversity of the donor and recipient samples revealed a significant sample type x microbiome treatment interaction (Table 1, Figure 10), and a significant main effect of both the sample type and microbiome treatment (Table 1). All pairwise comparisons for the main effects and the interaction effect on the combined data of recipients and donors were significantly different, except for the difference between the laboratory and natural inoculum treatment within the donors (Table S4). The analyses on beta diversity on the microbial donor inocula separately revealed no significant difference between the different inocula or microbiome treatments (Table
Bray-Curtis ordinations, however, demonstrated a complete separation between the natural and laboratory microbial donor inocula, indicating that the bacterial community of the inocula were differently structured (Figure S5). Analyses on beta diversity on the recipient’s gut microbial composition revealed that most of the variation was explained by the microbiome (lab versus natural) treatment (Table 1). The bacterial composition in recipients receiving the natural microbial inoculum differed significantly from those receiving the lab microbial inoculum (Table 1). Stressor, stressor x microbiome interaction and stressor x microbiome x genotype showed no significant contribution to the differences in beta diversity (Table 1). Ordinations based on Bray-Curtis, however, demonstrated an overlap between individuals exposed to natural and laboratory bacterial inoculum, indicating that the bacterial community of these communities were similarly structured (Figure 10). Separate analyses per microbiome treatment did not reveal a significant main effect of the stressor treatment in both *Daphnia* individuals that received a laboratory (R2=0.11379, df=3, p=0.694) or a natural microbial inoculum (R2=0.20147, df=3, p=0.18). Ordinations based on Bray-Curtis for *Daphnia* individuals that received the laboratory inoculum showed an overlap between all stressor treatments (Figure 11A). Ordinations based on Bray-Curtis for *Daphnia* individuals that received the natural inoculum, however, demonstrated a complete separation between the *Microcystis* and combi treatment, both showing small overlap with the control and infection treatment (Figure 11B) reflecting a specific *Microcystis* associated microbiome.
Figure 10: PCA of the gut microbial communities using weighted Bray-Curtis distance for donor and recipient data. Colors indicate microbiome treatment. Symbols and line type indicate sample type.

**Correlations**

Correlation tests were performed between percentage of survived *D. magna*, total brood, body size and OTU richness. No correlation was observed between the life history traits and OTU richness of the gut microbial community (Table S7, Figure S6). We did observe a positive correlation between survival and fecundity (cor=0.32, t= 2.84, df=70, p-adj=0.017; Table S5, Figure S7), and fecundity and body size (cor=0.33, t= 2.96, df=70, p-adj=0.017; Table S5, Figure S7).
Figure 11: PCA of the gut microbial communities using weighted Bray-Curtis distance for recipients exposed to (A) the lab microbial inocula, and (B) the natural microbial inocula. Colors indicate stressor treatment. Symbols indicate microbial inoculum.
Characterization of infection

After obtaining sequencing results (see Table S8), *Daphnia* with visible or non-visible infection showed the highest match with *Aspergillus aculeatus* and *Aspergillus niger*. Multiple sequence alignment further revealed a highly specific match with nucleotides 1 to 1900 for *Aspergillus aculeatus* KV879170 (strain: ATCC 16872, Figure S8). No specific match with *Aspergillus niger* was found in the multiple sequence alignment. Based on these results, we conclude that the fungal infection is related to *Aspergillus aculeatus* ATCC 16872.
The aim of this experiment was to investigate the response of *Daphnia* to either a single stressor or combination of both stressors when exposed to either a laboratory or a natural microbial inoculum. We inoculated germ-free *Daphnia* to either a laboratory or natural microbial community and compared host responses to a parasitic fungus, an *Aspergillus aculeatus* like strain, and the toxic cyanobacterium *M. aeruginosa* in single and combined exposures. Additionally, we examined intraspecific responses by including three *Daphnia* genotypes in our study. We expected that the gut microbiome (obtained after colonization by the inoculated bacterioplankton community), would affect stressor responses and alter interactions under multiple stressor conditions. We hypothesized that (i) *Daphnia* would obtain a higher tolerance to the stressors when receiving the natural microbial community compared with the laboratory microbial community and (ii) *Daphnia* genotype would shape responses to both single as multiple stressor exposures. Our results showed that (i) laboratory and natural microbiome communities differ in OTU richness, with a higher OTU richness in natural microbial communities, (ii) microbial exposure can alter stress responses for survival, but not for fecundity and body size, (iii) *Daphnia* responses are genotype dependent for survival and fecundity, but not for body size, and (iv) stressor specific microbiomes (*Microcystis* and combi) can be detected. Not all responses were in agreement with what we originally expected. More in particular, we only found an antagonistic response between both stressor treatments on *Daphnia* survival, and only when *Daphnia* were exposed to a laboratory and not to a natural microbial inoculum.
The results on OTU richness confirmed our assumption that natural microbial communities (obtained from natural freshwater waterbodies) are more diverse in number of strains compared with laboratory microbial communities (obtained from Daphnia medium). This more diverse community was also reflected in the gut of the Daphnia as the gut community of Daphnia individuals inoculated with a natural microbial inoculum had a higher OTU richness compared with those inoculated with a lab microbial inoculum. This is in accordance with other study systems (e.g. Drosophila: Chandler et al. 2011, Limulus polyphemus: Friel et al. 2020, zebrafish: Roeselers et al. 2011, mice: Rosshart et al. 2017). In addition, the OTU richness between donors and recipients significantly differed in the microbiome treatments. This suggests that Daphnia, when exposed to a rich bacterial community, selects certain bacterial strains from the bacterioplankton community, as suggested in Macke et al. (2017) and Mushegian et al. (2018) and shown in Callens et al. (2020). EdgeR analysis revealed that only one OTU differed between the laboratory and natural donor inocula. Combined with the lower OTU richness in the laboratory donor inocula compared with the natural ones, it appears that the laboratory microbial inocula contain a subset of the natural microbial inocula. The sampled laboratory donor inocula, thus, contained selected particular groups of bacteria from a diverse (natural) microbial inocula.

Analysis of the recipient microbiomes showed that selection of bacterial groups was also stressor dependent, with a stronger selection of bacterial strains in the Microcystis and combi treatment in Daphnia that received a natural microbial inoculum, as shown in the beta diversity analysis. This is conform as suggested in Macke et al. (2017, 2020) and Houwenhuyse et al. (2021). The microbial environment plays a role in stress responses when looking at Daphnia survival.

Survival analysis revealed a significant difference in the survival probability between the stressor
When Daphnia received a laboratory microbial inoculum, but not when they received a natural microbial inoculum. When receiving the laboratory microbial inoculum, survival probability was higher when exposed to both stressors simultaneously compared with exposure to these stressors separately. This suggests occurrence of an antagonistic interaction between the two stressors in relation to survival, resulting in a higher survival probability when exposed to both stressors compared with single stressor exposure. This is in accordance with the recent study of Boudry et al. (2020) who also observed an antagonistic interaction between a fungal infection (most likely the same as in this study) and Microcystis on Daphnia survival. Boudry et al. (2020) described this antagonistic interaction as a potential protective effect of the Aspergillus infection on Daphnia towards Microcystis exposure through a parasite-mediated reduction in toxicity of Microcystis. Alternatively, Daphnia can be boosted through an increase in general tolerance levels by ingestion of the produced zoospores. Cross-tolerance could be initiated as zoospores could function as an additional food source, which is in accordance with Frenken et al. (2017), Kagami et al. (2007) and Agha et al. (2016), indicating that fungal parasites can transfer energy and nutrients from otherwise inedible algae to Daphnia, and thereby increase Daphnia growth and survival. These studies, however, used fungal parasites that are obligate parasites from inedible diatoms and cyanobacteria. In this study, Aspergillus infects the Daphnia host, resulting in reduced body size and a genotype dependent reduction in fecundity, as well a high mortality in juveniles (L. Bulteel and S. Houwenhuyse, personal observations). It is, however, not yet examined whether this specific Aspergillus can also infect cyanobacteria. The Aspergillus genus is diverse and widespread containing up to 339 species (Samson et al. 2014), which consist of several pathogenic species, significantly impacting food production (e.g. Alshannaq et al. 2018), and animal and
human health (e.g. Kousha et al. 2011, Seyedmousavi 2013). *Aspergillus aculeatus* exposure, on the
other hand, has been described to be associated with phytoremediation and detoxification in
plants (Xie et al. 2019).

The antagonistic interaction on *Daphnia* survival was only observed when exposed to the less
diverse laboratory microbial community, which could imply a dependency on a particular,
selected and more effective (non-diluted in terms of number of strains present) microbiome. We
originally expected more positive effects on life history traits in the natural (e.g. Booth 2002, Van
Overbeek et al. 2010, van Elsas et al. 2012), more diverse microbial community, given the higher
potential for redundant effects, but here tend to find the opposite response. We here do not find
an antagonistic interaction in the multiple stressor treatment in the natural microbial treatment.
Given that the antagonistic interaction was only present in the laboratory treatment, it could
reflect that the presence of particular microbial strains that were more strongly selected for or the
phytoremediating effect of the fungus against *Microcystis* was not diluted by the presence of other
strains in the laboratory conditions. In this study, however, we found no possible correlation
between gut microbial diversity and the observed life history traits. We did find a differently
structured gut community within the laboratory treatment as *Daphnia* gut microbiomes exposed
to the multiple stressors showed a partial overlap with the communities exposed to all other
stressor treatments (control, *Aspergillus* and *Microcystis*), which already hints at a possible
microbiome-mitigated defense mechanism.

Exposure to a laboratory or a natural microbial community, however, had no impact on *Daphnia*
stress-responses for fecundity and body size. The antagonistic interaction between the stressors
observed in the survival analysis was not present for fecundity and body size. Both fecundity and body size did reveal a main stressor effect, independent of microbial exposure, showing a lower reproduction and body size for Daphnia that were exposed to the Microcystis and combi treatment compared with Daphnia that were exposed to the infection and control treatment. Body size, in addition, was lower for individuals exposed to Aspergillus compared with the control, whereas the opposite was observed for the total number of brood. Trade-offs between survival and body size under stress were previously found in Daphnia (Adamczuk 2010, Houwenhuyse et al. 2021) and other organisms (Sterck et al. 2006, Mogensen and Post 2012) with Houwenhuyse et al. (2021) suggesting a role of the microbiome for this trade-off under Microcystis stress. Here, we did, however, not find support for such a trade-off. Interestingly, both Microcystis and combi-exposed Daphnia show a stressor-specific microbiome, which could be a co-determinant of this lower body size and fecundity.

Survival and fecundity analyses revealed a role of the genotype in the stress responses, with for survival the genotype x stressor response being microbiome dependent. Body size, however, was not determined by genotype. The survival probability of genotype T8 was lower compared to KNO and OM2 when they were exposed to a control treatment and received a natural microbiome inoculum. No significant differences, however, were observed between the different genotypes under the control treatment when receiving a laboratory microbial inoculum. For fecundity, KNO and OM2 individuals reproduced a lower number of juveniles when they received a Microcystis or combi treatment compared with when they received the infection or control treatment. Fecundity of T8 individuals, however, was not differentially impacted by the different stressor treatments as no significant differences on the number of offspring were observed. When
examining the Union plots, we observed that for the control treatment, T8 had a higher number of unique and total OTUs, compared with KNO and OM2 when they received a natural microbial inoculum, but not when they received a laboratory microbial inoculum. This appears counterintuitive, but aquatic environments contain next to a plethora of beneficial and neutral bacterial strains, also obligate and opportunistic bacterial pathogens (Schulze et al. 2006), so it could be that with higher diversity more opportunistic microbiota are present (as also suggested in Callens et al. 2016). As Daphnia genotypes differ in their selective capacities to take up bacteria (Macke et al. 2017, Frankel-Bricker et al. 2020, Callens et al. 2020, Bulteel et al. 2021, Houwenhuyse et al. 2021), our results would suggest that genotype T8 might be less selective and takes up randomly also non-beneficial strains, at least in comparison with KNO and OM2.

In conclusion, laboratory conditions only contain a subset of the bacterial community from natural environments. We found that these different microbial conditions can affect stressor responses when looking at Daphnia survival. Antagonistic interactions between multiple stressors were present when Daphnia were exposed to a laboratory bacterial community, but not when exposed to a natural bacterial community. The microbial condition, however, did not play a role in determining Daphnia fecundity and body size. We did detect a main stressor effect for both fecundity and body size, with a lower reproduction and body size when exposed to the Microcystis and the combined stressor treatment compared with Daphnia that were exposed to the infection and control treatment. These effects could possibly be linked to the stressor specific microbiomes observed in the Microcystis and com bi treatment. In addition, stressor responses were genotype specific for survival and fecundity, which could be linked with different capabilities of the Daphnia genotypes to select beneficial or neutral microbial stains from the environment.
Materials and methods

Daphnia and algae culturing

To investigate the genotype effect, we used three different *D. magna* genotypes: KNO 15.04, OM2 11.3 and T8. The KNO 15.04 genotype (further referred to as KNO) was isolated from a small pond (350m²) in Knokke, at the Belgian coast (51°20'05.62"N, 03°20'53.63"E) and is the same clone as used in Macke et al. (2017, 2020). The OM2 11.3 genotype (further referred to as OM2) was isolated from a 3.7 ha inland pond located in Heverlee, in Belgium (50°51'45.0"N, 04°42'58.8"E) and was part of the clone set of Decaestecker et al. (2007). The T8 genotype was isolated from an 8.7 ha shallow, manmade pond, located in Oud Heverlee, Belgium (50°50'24.0"N, 04°39'40.4"E) and was part of the clone set of Cousyn et al. (2001). All clonal lineages were established from resting eggs, isolated from the lake sediment. All genotypes were maintained in the laboratory under standardized conditions for several years prior to the experiment. Stock *Daphnia* clonal lineages were cultured in filtered tap water at a temperature of 19 ± 1°C and under a 16:8h light:dark cycle in 2L glass jars (at a density of 20 individuals/L). They were fed three times per week with saturating amounts of the green algae *C. vulgaris*. The medium of the stock cultures was refreshed once per three weeks.

*Daphnia* were fed with *C. vulgaris* (strain SAG 211-11 B), which is considered as standard good-quality food for *Daphnia* (Munirasu et al. 2016). One of the stressors used in this experiment is the toxic cyanobacterial strain *M. aeruginosa* (strain PCC 7806), isolated from the Braakman reservoir in the Netherlands (51°19'22"N, 3°44'16"E) and part of the Culture Collections at Institute Pasteur (Paris, France). *Chlorella vulgaris* and *M. aeruginosa* were grown in WC medium (i.e. Wright’s
Cryptophyte medium) and modified WC medium (without Tris) respectively. The algae were cultured under sterile conditions in a climate chamber at 22 ± 1°C with a light:dark cycle of 16:8h in 2L glass bottles, with constant stirring and aeration. Filters (0.22 µm) were placed at the input and output of the aeration system to avoid any bacterial contamination. The algae were weekly harvested in the stationary phase. The axenity of the algal cultures was checked by sequencing and plating on LB- and R2A-plates.

Experimental design

With this experiment we aimed to investigate the impact of a natural versus a laboratory microbiome on the tolerance of D. magna individuals when exposed to two different stressors in single and combined exposures (Figure 12). Individuals, inoculated with either a natural or a laboratory microbial community, were exposed to one of the four following stressor treatments: an opportunistic infection (characterized as A. aculeatus, Figure 13A), a toxic cyanobacterium M. aeruginosa (Figure 13B), the combination of both Microcystis and the infection, and a control treatment (fed with C. vulgaris instead of Microcystis and no exposure to the infection). The opportunistic infection used in this experiment was characterized as a fungal Aspergillus aculeatus infection, which causes high mortality and reduced fecundity upon Daphnia individuals in the laboratory before, especially in (germ-free) juveniles. The infection is genotype specific (based on visual inspections and experience by the authors).
Figure 12: Experimental design. Axenic *Daphnia* individuals from three genotypes were exposed to a natural or laboratory microbial community (microbiome treatment). *Daphnia* individuals receiving a natural microbial community were exposed to filtered pond water. *Daphnia* individuals receiving a laboratory microbial community were exposed to filtered tap water originating from *Daphnia* stock cultures. All *Daphnia* individuals were then exposed to one of the four different stressor treatments: infection, *Microcystis*, combination of both *Microcystis* and infection or a control treatment. The experiment was performed in triplicate for each treatment and factor combination.
Figure 13: Microscopic pictures of the stressor treatments; (A) *Aspergillus* infection treatment: hyphae and surrounding spores stained with dapi with 400 x magnification under UV fluorescence (for characterization process, see further) and (B) *Microcystis* treatment: Colony of *Microcystis* surrounded with individual cells with 160 x magnification.

Each multifactorial combination of stressor treatment, microbiome treatment and genotype was replicated independently three times (independent maternal lines). To establish the independent maternal lines, three iso-female lines for each genotype were cultured in separate jars for at least two generations to control for maternal effects. These iso-female lines were kept in a mixture of filtered tap and pond water in a 9:1 ratio and fed every other day with a saturating amount of *C. vulgaris*. Medium was refreshed once per week. The first brood of the second generation was discarded, whereas eggs from the second brood were collected to obtain axenic (i.e. germ-free) juveniles following protocol from Bulteel et al. (2021) and Houwenhuyse et al. (2021).

The axenic individuals were then placed individually in a closed vial filled with 18 mL sterile filtered tap water and 2 mL of the corresponding microbiome treatment (natural or laboratory microbial community). The natural microbial communities were sampled from three local natural ponds. The laboratory microbial communities, on the other hand, were sampled from the medium.
from three different genotypes, which were cultured in the lab. In this manner, we were able to mimic bacterioplankton communities under natural (high bacterial diversity) and laboratory (low bacterial diversity) conditions. Each microbial community was subsequently filtered over 100 µm and 10 µm to remove debris. Each maternal line received one of the three natural or laboratory microbial communities (Table 2).

After receiving the corresponding microbial inoculum, the individuals remained in these conditions for 48h, allowing for the microbiota to colonize the Daphnia guts. On the third day, all individuals were fed with C. vulgaris (100*10³ cells/mL). On the fifth day, individuals were exposed to their corresponding stressor treatment (Figure 12). Individuals in the Microcystis treatment received a mixture of the toxic cyanobacterial strain M. aeruginosa and the non-toxic C. vulgaris in a 50:50 ratio on a daily base from day 5 onwards. Individuals in the infection treatment received a spore solution. The spore solution was obtained by squashing infected Daphnia individuals and was administered in a 1:3 ratio (1 infected individual per 3 to infect individuals). We assume little impact from the small bacterial community associated with the spore solution as administered volume is low and as administration occurred after the colonization of the microbial inocula (Vass and Langenheder 2017, Callens et al. 2020). Samples of the spore solution were sequenced to correct for contamination if necessary. Individuals in the combi treatment received both the spore solution on day 5 and the combination of the toxic M. aeruginosa and the non-toxic C. vulgaris in a 50:50 ratio from day 5 onwards. Individuals in the control treatment were not exposed to any stressor and were fed with C. vulgaris from day 5 onwards. After being exposed to their corresponding stressor treatment, the volume in the falcon tubes was gradually increased to 50 mL by adding 10 mL of sterile filtered tap water per day, and this for three consecutive days.
Food concentration in the first 6 days was low (100*10³ cells/mL) to ensure a sufficient stress response. From day 7 onwards, food concentration was increased to 200*10³ cells/mL. All individuals were monitored for survival and reproduction for 21 days. At the end of the experiment (day 21), the body size was measured (from top of the head to the base of the tail) and guts were dissected and collected per treatment in an Eppendorf tube filled with 10 µL of sterile MilliQ. Recipient guts were pooled per unique combination (4 stressor treatments x 2 microbiome treatments x 3 genotypes x 3 replicates). In addition, samples of the donor microbial inocula (n=6) were collected to compare bacterial communities.

Table 2: Overview of the origin of the microbial communities divided over the replicates.

<table>
<thead>
<tr>
<th>Replicate</th>
<th>Laboratory microbial inoculum</th>
<th>Natural microbial inoculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M5 lab medium</td>
<td>Kennedy pond (50°48'05.7&quot;N 3°16'33.0&quot;E)</td>
</tr>
<tr>
<td>2</td>
<td>T7 lab medium</td>
<td>Marionetten pond (50°47'43.5&quot;N 3°15'00.2&quot;E)</td>
</tr>
<tr>
<td>3</td>
<td>ZWE 2B lab medium</td>
<td>Kulak pond (50°48'30.8&quot;N 3°17'37.0&quot;E)</td>
</tr>
</tbody>
</table>

Library preparation and sequencing

To characterize the gut microbial communities from collected *Daphnia* guts, the guts of the surviving *Daphnia* per replicate were dissected under a stereo-microscope with sterile dissection needles at the end of the experiment and pooled per replicate (mean= 7.236 guts/sample; sd= 1.872 guts/sample; min= 2 guts; max= 10 guts; Table S6). Samples were stored under -20°C until further processing. DNA was extracted using a PowerSoil DNA isolation kit (MO BIO laboratories, Carlsbad, CA, USA). DNA was dissolved in 20 µL milliQ water. Because of initially low bacterial
DNA concentrations in some samples, a nested PCR was applied to increase specificity and amplicon yield. The full-length 16S rRNA gene was first amplified with EUB8F and 1492R primers on 10 ng of template using a high-fidelity SuperFi polymerase (Thermofisher, Merelbeke, Belgium) for 30 cycles: 98°C – 10 s; 50°C – 45 s; 72°C – 30 s. PCR products were subsequently purified using the CleanPCR kit (Qiagen, Antwerp, Belgium). To obtain dual-index amplicons of the V4 region, a second amplification was performed on 5 µL (=20-50 ng) of PCR product using 515F and 806R primers for 30 cycles: 98°C – 10 s; 50°C – 45 s; 72°C – 30 s. Both primers contained an Illumina adapter and an 8-nucleotide (nt) barcode at the 5'-end. For each sample, PCRs were performed in triplicate. Afterwards the PCR products were pooled and a small volume (5 µL) was loaded on a gel to check if the PCR amplified the correct fragment. The remaining volume of the PCR products were purified using the CleanPCR kit (Qiagen, Antwerp, Belgium). An equimolar library was prepared by normalizing amplicon concentrations with a SequalPrep Normalization Plate (Applied Biosystems, Geel, Belgium) and subsequent pooling. Amplicons were sequenced using a v2 PE500 kit with custom primers on the Illumina Miseq platform (KU Leuven Genomics Core), producing 2 x 250-nt paired-end reads. This way, 72 gut samples were generated representing 4 stressors x 2 microbiome inocula x 3 genotypes x 3 replicates. In addition, samples of the microbiome inocula (n=6), the stressor treatments (n=2) and C. vulgaris (n=1) were sequenced.

Life history traits data

Survival was analyzed using a log-rank or Mantel-Haenszel test. The survival times of individuals that were still alive at the end of the 21 day experiment were coded as right-censored. Normality
and skewness of body size and fecundity data were examined with Shapiro–Wilk test and

`ggqplot` function (package `ggpubr`). For fecundity and body size, we used the Akaike

information criterion (AIC) to select the best subset of variables to represent the best model. We

first evaluated to include maternal line as a random factor (with a linear mixed-effect model) or

not (with a general linear model). Secondly, we tested the significance of the fixed factors in the

model with the best random effects factor. Type II ANOVA tables for fixed-effect terms with

Satterhwaite and Kenward-Roger methods for dominator degrees of freedom for F-tests and p-

values were created (Anova function of the car package). Following the AIC criterium, a linear

mixed-effect model was chosen to evaluate fecundity and body size. In the final model, we

included microbiome treatment, stressor treatment and genotype as fixed factors, and maternal

line as random effect. We also included all possible interactions. Post hoc analysis were performed

using the ‘emmeans’ function with a ‘Tukey’ adjustment from the emmeans R package. All

statistical tests were performed in R 4.0.2 (R Core Team 2020).

*Daphnia* microbiome data

DNA sequences were processed following Callahan et al. (2016a). Sequences were trimmed (the

first 10 nucleotides and from position 180 onwards) and filtered (maximum of 2 expected errors

per read) on paired ends jointly. Sequence variants were inferred using the high-resolution

DADA2 method, which relies on a parameterized model of substitution errors to distinguish

sequencing errors from real biological variation (Callahan et al. 2016b). Chimeras were

subsequently removed from the data set. Taxonomy was assigned with a naïve Bayesian classifier

using the SILVA v132 training set. OTUs with no taxonomic assignment at the phylum level or
which were assigned as “chloroplast” or “cyanobacteria” were removed from the data set. After filtering, a total of 3 552 490 reads were obtained with on average 39 038.35 reads per sample, with most samples having more than 1000 reads. To visualize the bacterial families that differed between the treatments, OTUs were grouped at the order level, and orders representing <1% of the reads were discarded. Measures for \(\alpha\)-diversity of the recipient gut microbial communities within the different treatments (OTU richness) were calculated using the vegan package in R following Borcard et al. (2011). All samples were rarified to a depth of 1000 reads before analyzing \(\alpha\)-diversity. The effects of stressor treatment, microbiome treatment, genotype, and all possible interactions on OTU richness were assessed through a generalized linear model (GLM), assuming a Poisson distribution of the data and corrected for overdispersion. Maternal line was not included as a random factor as AIC criterium indicated that the model without inclusion of the maternal line was a better predictive model of the data. After testing the full model, we used the AIC criterium to select the best subset of variables to represent the best model. Pairwise comparisons among significant variables and their interactions were performed by contrasting least-squares means with Tukey adjustment. To examine differences in gut microbial community composition (\(\beta\)-diversity) among samples, a Bray-Curtis dissimilarity matrix was calculated and plotted using principal coordinates analysis with the phyloseq package in R. Multivariate community responses to treatments and genotype were investigated by means of Principal Coordinates Analysis. The effect of the stressor treatment, microbiome treatment, genotype, and all possible interactions on \(\beta\)-diversity were assessed through a permutation MANOVA, using the Adonis2 function in the vegan package in R. Obtained p-values were adjusted for multiple comparisons through the control of the false discovery rate (FDR). Pearson correlations were executed between the number
of sequenced guts and the OTU richness to check for interdependence. Stressor treatment, microbiome treatment, genotype, all two-way interactions, and the three-way interaction, all showed no significant correlation, dismissing the issue of interdependence (Table S7).

Additionally, correlation tests were executed between the different life history traits and the OTU richness of the gut microbial communities. Correlation coefficients and p-values were calculated for all examined correlations. Obtained p-values were adjusted for multiple comparisons through the control of the false discovery rate (FDR). To identify which bacterial classes significantly differed between the main effects and the interaction effects, relative abundances per order were calculated on the raw sequencing data, excluding the samples removed from the rarefaction.

Based on OTU presence, Union plots were created using the wilkox/unionplot function from GitHub, to show the unique and shared OTUs within and between the stressor x microbiome interaction. Additionally, differential abundance analyses were performed (edgeR function) on the raw sequencing data from which samples with less than 2 counts per million (CPM) in at least three samples were filtered out. All statistical tests were performed in R 4.0.2 (R Core Team 2020).

**Characterization infection**

To characterize the strain causing the infection in this experiment, samples of infected *Daphnia* with visible signs of the fungal infection and *Daphnia* with no visible infections were compared. Fifteen infected animals were transferred in whole per five individuals in a sterile Eppendorf tube. Guts from 60 infected animals were dissected and transferred per 20 guts to 10 µl of sterile MilliQ water. Samples were stored under -20°C until further processing. DNA of all samples was extracted using a PowerSoil DNA isolation kit (MO BIO laboratories). The total DNA yield was
determined using a Qubit dsDNA HS assay (Invitrogen) on 1 μL of sample. A PCR reaction was run using a combination of primers for the LSU and SSU region (see Table S9, White et al. 1990, Vilgalys and Sun 1994) on all of the template (98°C – 30s, 30 cycles of 98°C – 10s, 55°C – 45s, 72°C – 30s, and 72°C – 5s, 12°C hold) using the Platinum SuperFi DNA polymerase (Thermofisher).

PCR products were subsequently purified using the QIAquick PCR purification kit (Qiagen) and were sent for Sanger sequencing to LGC Genomics (Berlin, Germany). The sequences were first converted into consensus sequences using R (package BioCManager). As little similarity was obtained to develop the consensus sequences, non-consensus fasta files were used. The Basic Local Alignment Search Tool (BLAST), BLASTn was performed on the non-consensus fasta files, using FungiDB (Basenko et al. 2018). All query sequences were blasted with all the fungal species present in the database, including oomycetes. The Expectation value (E-value, expected number of hits) was set as 50% of the length of the query sequence. Maximum descriptions (number of descriptions/alignment to show) were set to 50 to avoid compromising the e-value and possible sequence matches. Additionally, the low complexity filter mode was set off to avoid omittance of results which contain repetitive and low complexity sequences. Similar settings were performed for all blasted sequences. Obtained results of fungiDB were verified using NCBI, emboss and wasabi. For NCBI the BLASTn protocol was followed (Schoch et al. 2014). To improve the sequence matches with Fungi, BLAST search was limited to RefSeq sequences only (using BioProject Number specific to Fungi, 177353, Schoch et al. 2014). Furthermore, emboss, with the Emboss matcher algorithm, was used to create the pairwise alignment between the sequences using the BLOSUM 62 matrix (Rice et al. 2000). Finally, a reference based multiple sequence alignment was performed to create a multiple sequence alignment table, using PRANK.
(probabilistic multiple alignment program for DNA) hosted by wasabi using the HKY model
(Veidenberg et al. 2015). The results were consistent across all databases (FungiDB, NCBI, emboss
and wasabi).

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Data availability

The datasets and scripts generated for this study can be found in the NCBI, under accession
number PRJNA731313 and on Zenoda with DOI: 10.5281/zenodo.4778716.

Conflict of interest

The authors declare that there is no conflict of interest.
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Figure S1: Survival plots recipient *Daphnia* between the stressors treatments for (A) the lab microbial inocula and (B) the natural microbial inocula. Colors indicate the different stressor treatments.
Figure S2: Boxplots of the total brood for the different stressor treatments. Colors indicate the different stressor treatments.
Figure S3: Boxplots of the total brood for the different stressor treatments under the different microbial inocula treatment. Colors indicate the different stressor treatments.
Figure S4: Unionplots representing the OTUs that are unique within and shared between (A) the control treatment and single stressor treatments and (B) the single treatments and the multiple stressor treatment. OTUs are illustrated for A and B: the control treatment (C), infection treatment (F) and Microcystis treatment (M) and C and D: infection treatment (F), Microcystis treatment (M) and combi treatment (MF). Numbers between brackets indicate OTUs present in that compartment. Colors indicate the class level per OTU.
Figure S5: PCA of the (A) donor and (B) recipient microbial communities using weighted Bray-Curtis distance. Colors indicate the different microbiome treatments.
Figure S6: Pearson regression between (A) Survival, (B) Fecundity, (C) Body size and OTU richness of the gut microbial community of recipient *Daphnia*. Non-adjusted p-values and correlation coefficient (R) are noted per figure.
Figure S7: Pearson regression between (A) Fecundity and body size, and (B) Survival and Fecundity. Non-adjusted p-values and correlation coefficient (R) are noted per figure.
Figure S8: Part (155 to 310 nucleotides) of the multiple sequence alignment pattern of the sample sequences with *Aspergillus aculeatus* ATCC 16872. Sequences of *Daphnia* with a visible and no visible infection, together with the *Aspergillus aculeatus* ATCC 16872 strain are aligned (sample names are shown in the left column). Color represents a specific type of nucleotide that matches with the *Aspergillus aculeatus* strain. Hyphen (-) represents a gap where no match between the nucleotides of the *Aspergillus aculeatus* strain and the aligned sequence of the sample is found. Asterisk on the top represents the nucleotides that are common in all the aligned sequences.